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Mild COVID-19 infection does not alter the ovarian reserve in women treated with ART

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ABSTRACT

Research Question

Does mild COVID-19 infection impact the ovarian reserve of women undergoing an Assisted Reproductive Technology (ART) protocol?

Design

We conducted a prospective observational study between June and December 2020. We included women managed in our ART unit for fertility issues by in vitro fecundation / intracytoplasmic sperm injection (IVF/ICSI), fertility preservation (FP), frozen embryo transfer (ET) or artificial insemination (AI) and with an AMH test performed within 12 months preceding ART treatment. All the women underwent a COVID rapid detection test (RDT) and we compared AMH levels between those who tested positive (RDT+) and those who tested negative (RDT-).

Results

The study population consisted of 118 women, 11.7% (14/118) of whom were COVID RDT+. None of the tested women presented with a history of severe COVID-19 infection. The difference between the initial AMH level and AMH level tested during ART treatment was comparable between the COVID RDT+ group and COVID RDT- group [-1.33 (-0.35 – -1.61) versus -0.59 (-0.15 – -1.11), p=0.22].

Conclusion

Our study suggests that a history of mild COVID-19 infection does not seem to alter the ovarian reserve as evaluated by AMH levels. While these results are reassuring, further studies are necessary to assess the impact of COVID-19 on pregnancy outcomes in women undergoing ART.

KEY WORDS:

COVID; ovarian reserve; ART; AMH

Abbreviations

ACE2 - angiotensin-converting enzyme 2

AMH – anti-mullerian hormone

ART - Assisted Reproductive Technology

RDT - rapid detection test

INTRODUCTION

Since December 2019, the world has been facing a COVID-19 pandemic. Besides its impact on the mortality, COVID-19 infection raises questions about short- and long-term effects on general health. Clinical manifestations are highly heterogeneous and involve many different organs (Lai et al., 2020).

The SARS-CoV-2 virus penetrates human cells by directly binding with angiotensin-converting enzyme 2 (ACE2) receptors present on the cell surface (Bornstein et al., 2020). ACE2 receptors are present in testis (Fan et al., 2020; Fu et al., 2020; Stanley et al., 2020) and in ovarian tissue (Jing et al., 2020; Reis et al., 2011; Stanley et al., 2020). In the ovary, ACE2 plays a role in the response to gonadotropins, steroidogenesis regulation, and in follicle development, angiogenesis and degeneration (Domińska, 2020; Jing et al., 2020). It has been suggested that SARS-CoV-2 could be responsible for testicular lesions (Fan et al., 2020). Analysis of testicular specimens from autopsies of men who died from COVID-19 showed modifications of the testicular structure – a thickening of the basal layer of seminiferous tubules, a decrease or absence of spermatozoa, decrease in the number of Leydig cells, lymphocyte infiltration, and germinal cell degeneration – compared with matched controls who died from other pathologies (Chen and Lou, 2020; H. Li et al., 2020; Ma et al., 2021; Yang et al., 2020). Testicular pain has been reported in about 20% of men with COVID-19 infection (Pan et al., 2020).

SARS-CoV-2 RNA was not found in the follicular fluid of two women who tested positive for COVID-19 and who were undergoing controlled ovarian hyperstimulation for in vitro fertilization (IVF) (Barragan et al., 2020). However, the modification of ovarian reserve by COVID-19 infection has not been evaluated to date.

Thus, the objective of this prospective study was to evaluate the impact of mild COVID-19 infection on the ovarian reserve in women undergoing an Assisted Reproductive Technology (ART) protocol.

MATERIEL AND METHODS

Study Population

This single-centre prospective observational study was conducted in the ART unit of Tenon Hospital, Paris between June 2020 and December 2020.

Women aged 18-43 years managed for fertility issues by IVF / intracytoplasmic sperm injection (IVF/ICSI), fertility preservation (FP), frozen embryo transfer (ET), or artificial insemination (AI) with an initial AMH level tested within the 12 months preceding ART treatment, were invited to participate in the study.

Data collection

Demographic characteristics including age, body mass index (BMI, kg/m^2), tobacco smoking, presence of insufficient ovarian reserve (IOP), endometriosis, fallopian tube pathology and initial AMH level were retrieved from a prospective database. The type of ART protocol, the time between the initial (baseline) and second AMH test as well as the oestradiol level on the day of AMH test were also recorded.

As recommended by the French Agency of Biomedicine (*Agence de Biomédecine),* all the women completed a questionnaire about any COVID-19 infection symptoms that may have occurred during the 2 weeks prior to ART treatment. COVID-19 serology status was tested on the first day of ovarian stimulation monitoring with a COVID-19 rapid detection test (RDT) kit (UNCOV-40, Clinisciences, France) according to the manufacturer's instructions. Ovarian reserve was evaluated by AMH tested on the day of ovarian stimulation monitoring and ovarian reserve modification was calculated by the difference between the baseline AMH level (tested within the preceding 12 months) and this new AMH level.

All women included in the study expressed non-opposition consent to participate in the study. The procedures used in the study were in accordance with the guidelines of the Helsinki Declaration on Human Experimentation and the Good Clinical Practice (CGP) and approved by the IRB (CEROG 2021-GYN-0508).

Statistical analysis

Quantitative variables are presented as means with standard deviation (SD) or medians with interquartile range (IQR) as appropriate. Qualitative variables are expressed as numbers with percentages (%). Differences in population characteristics between COVID+ and COVID- women were evaluated with Student's-t test / Mann-Whitney test or Chi2 / Fisher exact test as appropriate. The difference in AMH levels between the COVID RDT+ and COVID RDT- women was evaluated with the Mann-Whitey test.

All tests were two-sided and $p<0.05$ was considered to be statistically significant. Analyses were done with Prism 7.

RESULTS

Population characteristics

Of the 960 women who underwent an ART protocol in our unit during the study period (June 2020 and December 2020), 118 accepted to participate in the study. The prevalence of COVID RDT+ in the tested population was 11.7% (14/118). None of the women included in the study presented clinical manifestations of COVID during the 2 weeks preceding the beginning of the ART protocol. Neither had any of the women presented the severe form of COVID-19 infection or required hospitalisation during the pandemic period.

The characteristics of the women with COVID RDT+ and COVID RDT- are presented in Table 1. There was no difference in age, BMI, tobacco smoking, infertility aetiology, baseline AMH level, or ART protocol type. The time between the baseline and second AMH test was comparable between the two groups as was the oestrogen level on the day of AMH test.

The median level of AMH tested during ART treatment was comparable between the two groups: 1.51 (0.0.82-2.38) in COVID RDT+ group versus 1.00 (0.49-1.99) in COVID RDT- group (p=0.27).

Similarly, the difference between the baseline and second AMH levels was comparable between the groups: -1.33 (-0.35 – -1.61) in COVID RDT+ group versus -0.59 (-0.15 – -1.11) in COVID RDT- group, (p=0.22).

DISCUSSION

The results of this prospective study showed that, based on AMH levels, mild COVID-19 infection did not impact the ovarian reserve in our population of asymptomatic women who underwent an ART protocol in our unit. The baseline AMH level, the level tested during the ART treatment, as well as the difference between the two AMH levels, were comparable between the COVID RDT+ group and COVID RDT- group.

To date, the total number of confirmed cases of COVID-19 infection worldwide is about 71 500 000 ("https://www.santepubliquefrance.fr/dossiers/coronavirus-covid-19/coronavirus-chiffres-cles-et-evolution-de-la-covid-19-en-france-et-dans-le-monde," 2020) which represents 0.09% of the population overall. In France, there are 2 500 000 confirmed cases ("https://www.santepubliquefrance.fr/dossiers/coronavirus-covid-19/coronavirus-chiffres-cles-et-evolution-de-la-covid-19-en-france-et-dans-le-monde," 2020) which represents 3.7% of the French population overall and about 5% of the adult population. The prevalence of positive COVID RDT+ in our study population was high at 11.7%. This can be explained by the fact that serology testing is not offered systematically in France, and many asymptomatic cases remain undetected. Thus, the number of COVID-19 cases in the general population is certainly underestimated.

While none of included women reported having symptoms of COVID-19 in the 2 weeks preceding their ART treatment, it is not known if they presented minor symptoms of COVID-19 infection earlier on. However, none of the women had presented the severe form of COVID-19 infection requiring hospitalisation during the pandemic period.

The extra-respiratory manifestations of COVID-19 are diverse and involve multiple organs (Lai et al., 2020). It has been suggested that COVID-19 infection could impact the female reproductive system, as the virus enters target cells by interacting with ACE2 receptors which are expressed in the ovaries (Jing et al., 2020; Singh et al., 2020). Other viral infections, such as HIV or viral hepatitis, have been shown to potentially alter ovarian reserve (Kurmanova et al., 2016; Santulli et al., 2016; Seifer et al., 2007).

Li *et al.* demonstrated that sex hormone concentrations and AMH levels in women of reproductive age hospitalised for confirmed COVID-19 infection were comparable to the age-matched controls, even if 28% of the COVID-19 positive women in their study presented changes in their menstrual cycle and 25% changes in their menstrual volume (K. Li et al., 2020). Our study confirms these results. The median level of AMH in the COVID RDT+ women was comparable with that found in the COVID RDT- women ($p=0.27$). Moreover, the difference between two AMH levels tested in the same women at different times was comparable between both groups ($p=0.22$).

In contrast to the study by Li *et al.* (K. Li et al., 2020), which was performed in a population of hospitalised women, we evaluated mid- and long-term effects of COVID-19 infection on ovarian reserve: AMH levels were tested during ART treatment some time after a potential COVID-19 infection and in women without or with few symptoms.

We evaluated COVID-19 infection by SARS-CoV-2 serology using an immunochromatographic assay. This method is characterized by high specificity and sensitivity (98.02% and 98.81%, respectively, according to the manufacturer). However, it is not clear as yet how long antibodies persist after COVID-19 infection (Milani et al., 2020).

The strength of our study is that we tested AMH levels in the same women at different time points and could thus analyze any potential modification of the ovarian reserve after COVID-19 infection.

However, the study has some limitations. Firstly, it has been shown that the AMH level is modified during ART treatment (Peñarrubia et al., 2005) as this hormone is secreted by granulosa cells of small growing follicles (Moolhuijsen and Visser, 2020) thus reflecting rather the granulosa cell activity. Nevertheless, the baseline and the second AMH levels, as well as ART protocol types and oestrogen levels on the day of AMH testing were comparable between both study groups. Secondly, a relatively small number of women were included in the analysis and only 14 were COVID RDT+. In addition the group with COVID-19 RDT+ and the control group were heterogenous due to the methodology of inclusion consisting in a consecutive patients' inclusion. ART treatments in our unit were postponed for 3 months during the COVID epidemic following interruption of activity due to a decision by the French government. Thus, the time between the baseline AMH test and the beginning of the ART treatments were extended. However, we decided to include only women with AMH tested within the 12 preceding months because AMH level is age dependent and can decrease over time (Plociennik et al., 2018). The time between the two AMH tests was comparable between the study groups.

CONCLUSION

In conclusion, our study suggests that a history of mild COVID-19 infection does not seem to alter the ovarian reserve. Even if these results are reassuring, further studies especially with larger samples are required to confirm our findings.

AUTHOR CONTRIBUTION

KK, JMA, ED and NCB were involved in conception and design, KK, AH, LJ, EMA and CD were involved in acquisition of data and KK, YD, CT, JMA, ED and NCB were involved in data analysis and interpretation. KK, AH, LJ, ED and NCB were involved in drafting the manuscript and all authors were involved in revising the manuscript critically for important intellectual content. All authors have given final approval for submission.

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DECLARATIONS OF INTEREST

None

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KEY MESSAGE

Mild COVID-19 infection did not alter the ovarian reserve in women treated with ART. The baseline AMH, AMH tested during the ART treatment, as well as the difference between the two AMH levels, were comparable between the COVID(RDT)+ and COVID(RDT)- groups.

Table 1. Characteristics of the study populations.

	COVID RDT+ (n = 14)	COVID RDT- (n = 104)	p value
Age (years), mean (SD)	35.7 (4.2)	34.5 (4.5)	0.32
BMI (kg/m^2), mean (SD)	23.1 (3.7)	24.3 (5.5)	0.29
Tobacco smoking, n (%)	2 (14)	18 (17)	0.78
IOP, n (%)	1 (7)	22 (21)	0.21
Endometriosis, n (%)	3 (21)	30 (29)	0.56
Fallopian tube pathology, n (%)	4 (29)	14 (13)	0.14
Initial AMH level (ng/mL), median (IQR)	2.87 (1.69-3.99)	1.76 (0.88-3.00)	0.13
Period between the two AMH tests (days), median (IQR)	254 (211-285)	268 (158-327)	0.77
ART protocol			0.38
IVF/ICSI, n (%)	11 (79)	64 (62)	
FP, n (%)	0 (0)	17 (16)	
AI, n (%)	2 (14)	18 (17)	
ET, n (%)	1 (7)	5 (5)	
Oestrogen level on AMH testing day (ng/mL), median (IQR)	718 (239-1534)	664 (277-1353)	0.87

AI – artificial insemination
AMH – Anti-Müllerian Hormone
ART – Assisted Reproductive Technology
BMI – body mass index
ET – embryo transfer
FP – fertility preservation
IOP – insufficient ovarian reserve
IQR – interquartile range
IVF / ICSI – in vitro fecundation / intracytoplasmic sperm injection
RDT – rapid diagnostic test
SD – standard deviation

Table 2. Anti-Müllerian hormone (AMH) levels in COVID rapid diagnostic test (RDT) positive and COVID RDT negative groups.

	COVID RDT+ (n = 14)	COVID RDT- (n = 104)	p value
New AMH level (ng/mL), median (IQR)	1.51 (0.82-2.38)	1.00 (0.49-1.99)	0.27
ΔAMH (ng/mL), median (IQR)	-1.33 (-0.35 – -1.61)	-0.59 (-0.15 – -1.11)	0.22